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Genetic variability of DNA repair mechanisms in chemotherapy treatment outcome of gastric cancer patients

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ABSTRACT. We investigate whether three common polymorphisms in *ERCC1* and *ERCC2* are predictor factors for the chemotherapy response, as well as the clinic outcome of patients with gastric cancer. Between May 2011 and May 2013, 263 patients with gastric cancer who were newly diagnosed by histopathology were enrolled in our study. Genotyping of the *ERCC1* rs11615 and rs3212986, and *ERCC2* rs1799793 polymorphisms were conducted by the polymerase chain reaction-restriction fragment length polymorphism assay. Patients carrying the TT genotype and TT+CT genotype of *ERCC1* rs11615 were associated with poorer response to chemotherapy and shorter survival times when compared with the CC genotype. In conclusion, our results suggested that the *ERCC1* rs11615 polymorphism in the DNA repair pathways can be used as predictive factors to the clinical outcome of patients with gastric cancer.

Key words: ERCC1; ERCC2; Polymorphism; Overall survival; Response to chemotherapy; Gastric cancer

INTRODUCTION

Gastric cancer remains a severe public health problem worldwide. It is estimated that there are 989,600 new patients with gastric cancer and 738,000 deaths every year because of this cancer (International Agency for Research on Cancer, 2012). Gastric cancer is often diagnosed until reaching the advanced clinical stage, characteristic of obvious lymphatic tumor dissemination (Boddie et al., 1989).

Postoperative neoadjuvant chemotherapy is the main modality for chemotherapy to improve the survival time of advanced gastric cancer patients. Despite improved protocols for diagnosis and treatment, gastric carcinoma still has a poor prognosis. Following curative surgical resection and neoadjuvant chemotherapy, 5-year survival of gastric cancer is between 10-15% (Stadtlander and Waderbor, 1999). Previous studies have suggested that genetic polymorphisms are involved in the individualized therapy as well as in the treatment outcome of gastric cancer patients (Li et al., 2013; Lu et al., 2014).

The DNA repair systems play an important role in repairing the damage to DNA induced by endogenous and/or exogenous factors such as therapeutic agents. The nucleotide excision repair (NER) pathway is a versatile system to monitor and repair DNA damage, and the alternation of NER capacity could play a pivotal role in the clinical outcomes of gastric cancer patients.

The excision repair cross-complimentary group 1 (ERCC1) and group 2 (ERCC2) proteins are two important endonucleases in the NER pathway, whose products are important in NER on chromosome 19q13.3. Recently, several studies have focused on the association between ERCC1 and ERCC2 gene polymorphisms and clinical outcome of several kinds of cancers (Kumamoto et al., 2013; Nishina et al., 2013; Henríquez-Hernández et al., 2014; Li et al., 2014a; Sullivan et al., 2014; Zhou et al., 2015). However, only a few studies have reported on the role of ERCC1 and ERCC2 expression in the clinical outcome of patients with gastric cancer treated with chemotherapy (Yin et al., 2011; Li et al., 2013). Here, we investigate whether three common polymorphisms in *ERCC1* and *ERCC2* are predictor factors for the chemotherapy response, as well as the clinic outcome of patients with gastric cancer.

MATERIAL AND METHODS

Patients

A total of 291 patients with gastric cancer who were newly diagnosed by histopathology were recruited from the Tianjin Nankai Hospital and Tianjin Medical University Cancer Institute Hospital between May 2011 and May 2013. The exclusion criterion were patients who had recurrent tumors, were pregnant, or had organ failure. Finally, 263 patients with gastric cancer agreed to participate in our study, and the participation rate was 90.38%. This study project was approved by the Ethics Committee of Tianjin Nankai Hospital and the written inform consents were obtained from all patients.

All included patients were followed-up until May 2014. All patients were followed up by telephone or through attending clinics every 4 weeks until death or the end of the follow-up period.

Assessment of treatment outcome

All patients received FOLFOX chemotherapy. Tumor response to chemotherapy was evaluated by World Health Organization (WHO) criteria (Miller et al., 1981): complete response

or partial remission were good response to chemotherapy, whereas stable or progressive disease were poor response to chemotherapy. The overall survival time (OS) was calculated from the date of enrollment into our study to the date of death or the end of follow-up.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini kit (Qiagen, Valencia, CA, USA). Genotype determination of *ERCC1* rs11615 and rs3212986, and *ERCC2* rs1799793 were conducted by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. Probes and primers for *ERCC1* rs11615 and rs3212986, and *ERCC2* rs1799793 were designed using the Sequenom Assay Design 3.1 software (Sequenom®, San Diego, CA, USA). Forward and reverse primers for *ERCC1* rs11615 were 5'-GCAGAGCTCACCTG AGGAAC-3' and 5'-GAGGTGCGGCGGGACCTAAA-3', respectively. Forward and reverse primers for *ERCC1* rs3212986 were 5'-CGAACCGAGTGGGCCAAGAG-3' and 5'-GCCCAGGTTCAGCGG CCTTT-3', respectively. Forward and reverse primers for *ERCC2* rs1799793 were 5'-CAGCTCATG TCTGGGCACCATCAA-3' and 5'-GTCGGCCCTCACGGTGCACGAGTTGCT-3', respectively. The restriction enzymes for *ERCC1* rs11615 and rs3212986, and *ERCC2* rs1799793 were *Tai, Mbo*II and *Eco*130I. PCR cycling was performed at 50°C for 2 min, 95°C for 10 min, 94°C for 20 s, and then 62°C for 60 s for 35 cycles. Enzyme digestion followed the identification process. The PCR products were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized under UV light. A fraction (5%) of the blood samples was randomly selected for re-ascertainment to maintain genotyping quality, and the results of repeated samples showed 100% concordance.

Statistical analysis

Continuous variables are reported as means ± standard deviation (SD) and categorical variables are reported as number (N) and percentage (%). The association between *ERCC1* rs11615 and rs3212986, and *ERCC2* rs1799793 polymorphisms and the response to chemotherapy was assessed by the conditional logistic regression analysis, and the results are reported by ORs and 95%CIs. The association between the three gene polymorphisms and OS of patients with gastric cancer was evaluated by the Cox proportional hazard model to assess hazard ratios (HRs) and 95%CIs.

Statistical analysis was conducted using the SPSS 17.0 statistical software (SPSS, Chicago, IL, USA). P values set at <0.05 were considered as representing a statistical difference.

RESULTS

A total of 263 patients including 173 (65.78%) males and 90 (34.22%) females were enrolled in the present study (Table 1). The mean age of gastric cancer patients was 62.40 ± 9.50 years. At the last follow-up, 152 (57.79%) patients represented good response to chemotherapy, and 111 (42.21%) presented poor response. Among the patients, 119 (45.25%) patients were at stages I-II and 144 (54.75%) were at stages III-IV; 101 (38.40%) patients were intestinal type and 162 (61.60%) were diffuse type; 99 (37.64%) patients were lymphatic metastasis positive while 164 (62.36%) patients were negative.

Table 1. Demographic and clinical characteristics of the patients included.

Characteristics	Patients (N)	Percent (%)
Gender		
Male	173	65.78
Female	90	34.22
Age (years)		
<60	114	43.35
≥60	149	56.65
TNM stage		
I or II	119	45.25
III or IV	144	54.75
Histological type		
Intestinal	101	38.40
Diffuse	162	61.60
Lymphatic metastasis		
Negative	99	37.64
Positive	164	62.36
Response to chemotherapy		
Good	152	57.79
Poor	111	42.21

TNM = tumor, lymph node, metastasis.

In a logistic regression model, after adjusting for potential confounding factors, patients carrying the TT genotype of *ERCC1* rs11615 showed a significantly poorer response rate than did those with the CC genotype, and the adjusted OR (95%CI) was 0.41 (0.19-0.88) (Table 2). In addition, subjects carrying the TT+CT genotype of ERCC1 rs11615 showed a poorer response rate than did those with the CC genotype; the adjusted OR (95%CI) was 0.55 (0.32-0.94). However, the *ERCC1* rs3212986 and *ERCC2* rs1799793 polymorphisms were not associated with response to chemotherapy in patients with gastric cancer (Table 2).

Table 2. Association between *ERCC1* and *ERCC2* gene polymorphisms and response to chemotherapy in patients with gastric cancer.

Genotype	Good response (N = 152)	Percent (%)	Poor response (N = 111)	Percent (%)	Adjusted OR (95%CI)[1]	P value
ERCC1 rs11615						
CC	74	48.68	38	52.81	1.00 (ref)	-
CT	57	37.50	47	34.83	0.62 (0.35-1.11)	0.09
TT	21	13.82	26	12.36	0.41 (0.19-0.88)	0.01
CT+TT	78	51.32	73	47.19	0.55 (0.32-0.94)	0.02
ERCC1 rs3212986						
CC	74	48.68	61	55.06	1.00 (ref)	-
CA	53	34.87	36	33.71	1.21 (0.68-2.17)	0.48
AA	25	16.45	14	11.24	1.47 (0.67-3.34)	0.3
CA+AA	78	51.32	50	44.95	1.29 (0.76-2.17)	0.32
ERCC2 rs1799793						
GG	86	56.58	57	47.19	1.00 (ref)	-
AG	57	37.50	45	42.70	0.84 (0.49-1.45)	0.51
AA	9	5.92	9	10.11	0.66 (0.22-2.02)	0.41
AG+AA	66	43.42	54	52.81	0.81 (0.48-1.36)	0.4

[1]Ajusted for age, gender, histological type, lymphatic metastasis and TNM stage.

In our cohort, 85 patients died from gastric cancer during the follow-up period; thus, the OS rate was 67.67%. Using Cox proportional hazard analysis, we found that individuals carrying the TT genotype of *ERCC1* rs11615 had a higher risk of death when compared with those carrying the CC genotype, and the HR (95%CI) was 2.72 (1.22-6.01) (Table 3). Individuals carrying the T

allele of *ERCC1* rs11615 were also associated with an increased risk of death when compared with those carrying the CC genotype (HR = 1.87, 95%CI = 1.03-3.44) when compared with those carrying the AA genotype. However, we found no association between the *ERCC1* rs3212986 and *ERCC2* rs1799793 polymorphisms and overall survival in patients with gastric cancer (Table 3).

Table 3. Association between gene polymorphisms and overall survival in patients with gastric cancer.

Genotype	Deaths (N = 75)	Percent (%)	Alive (N = 178)	Percent (%)	Adjusted HR (95%CI)[1]	P value
ERCC1 rs11615						
CC	24	32.00	88	49.44	1.0 (Ref.)	
CT	31	41.33	73	41.01	1.56 (0.80-3.03)	0.16
TT	20	26.67	27	15.17	2.72 (1.22-6.01)	0.01
CT+TT	51	68.00	100	56.18	1.87 (1.03-3.44)	0.03
ERCC1 rs3212986						
CC	36	48.00	99	55.62	1.0 (Ref.)	
CA	26	34.67	63	35.39	1.13 (0.60-2.14)	0.68
AA	13	17.33	26	14.61	1.38 (0.58-3.13)	0.41
CA+AA	39	52.00	89	50.00	1.21 (0.68-2.13)	0.49
ERCC2 rs1799793						
GG	38	50.67	105	58.99	1.0 (Ref.)	
GA	30	40.00	72	40.45	1.15 (0.63-2.10)	0.62
AA	7	9.33	11	6.18	1.76 (0.53-5.38)	0.27
GA+AA	37	49.33	83	46.63	1.23 (0.69-2.18)	0.45

[1]Ajusted for age, gender, tumor histology, and TNM stage. HR = hazard ratio.

DISCUSSION

In the present study, we investigated the role of *ERCC1* rs11615 and rs3212986, and *ERCC2* rs1799793 polymorphisms in the treatment response or OS in patients with gastric cancer treated with chemotherapy. Our analysis indicated that the TT genotype and CT+TT genotype of *ERCC1* rs11615 were significantly associated with poorer response to chemotherapy when compared to the CC genotype, and that these genotypes were also associated with short OS of patients with gastric cancer.

It is well known that the NER process plays an important role in damage recognition, damage demarcation and unwinding, damage incision, and new strand ligation (Ng et al., 2003; Kamileri et al., 2012). Many genes are involved in the NER pathway and in charge of different functions. ERCC1 is involved in the DNA damage incision, and ERCC2 is responsible for the damage unwinding process. The polymorphisms in ERCC1 and ERCC2 could alter the NER capacity, and thus change frequencies of DNA mutation due to unpaired damaged DNA. Therefore, the polymorphisms in NER genes may affect the OS of patients with gastric cancer.

The ERCC1 enzyme belongs to NER system, which has the ability of repairing DNA adducts and other DNA helix-distorting lesions (de Laat et al., 1999), including platinum intra-strand DNA adducts, and it is considered to be related to resistance to platinum-based chemotherapy through reducing platinum-induced DNA damage (Kwon et al., 2007; Ceppi et al., 2008). Several studies have reported the role of ERCC1 polymorphisms in the treatment outcome of patients with gastric cancer, but the results are controversial (Chen et al., 2014; Li et al., 2014b; Liu et al., 2014; Lu et al., 2014; Zhou et al., 2015). Chen et al. (2014) reported that the *ERCC1* rs11615 TT genotype had increased risk of death from gastric cancer. Li et al. (2014) reported that *ERCC1* rs11615 polymorphisms were associated with risk of death when compared with the wide-type genotype. Liu et al. (2014) reported that carriers of the *ERCC1* rs11615 TT genotype had a higher increased risk of OS when compared with CC genotype. Zhou et al. (2015) suggested that the TT genotype of *ERCC1* rs11615 was significantly associated with a short OS of gastric cancer.

However, Lu et al. (2014) had indicated that the T allele of *ERCC1* rs11615 could decrease a 0.62-fold risk of death from gastric cancer. In our study, we found that the TT genotype of *ERCC1* rs11615 was associated with poor response to chemotherapy and short OS of patients with gastric cancer. The differences between the results in these studies might be caused by variations in sample size, ethnicity, and source of subjects.

Two limitations should be considered in this study. First, selection bias might exist in our study, since patients were selected from a single hospital. Second, the sample size of our study is relatively small, which might decrease the statistical power and cause statistical bias. Therefore, further large sample and multi-ethnic studies are greatly needed to confirm our results.

In conclusion, our results suggested that the *ERCC1* rs11615 polymorphism in the DNA repair pathways can be used as predictive factors to the clinical outcome of patients with gastric cancer. Further large sample and multicenter studies involving various populations are required to confirm our findings.

Conflicts of interest

The authors declare no conflict of interest.

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